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Noncultivable Viruses and Neonatal Diarrhea: Fifteen-Month Survey in a Newborn Special Care Nursery

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During a 15-month period of surveillance, diarrhea developed in 257 of 913 babies (28%) admitted within 2 hours of birth to a special care nursery in Melbourne, Australia. Diarrhea was seasonal, affecting a maximum of 43% of babies admitted during one winter month (July) and a minimum of 13% of babies admitted during one summer month (December). Diarrhea was no more frequent nor more severe in babies of very low birth weight or of very early gestational age. Two noncultivable viruses were located by electron microscopy in feces from babies with or without diarrhea. Excretion of a reovirus-like particle (rotavirus, duovirus, human reovirus-like agent, infantile gastroenteritis virus) was temporally related to diarrheal symptoms. Asymptomatic infection with this virus also occurred. A 28-nm virus-like particle was excreted by some babies, but it could not be implicated on epidemiological grounds in the etiology of the diarrhea. Rotavirus infection may be an important cause of endemic diarrhea in nurseries for the newborn. Infection may be difficult to control or eradicate, since it is often asymptomatic and may be influenced by infection in the community at large.

Infection is a serious hazard in neonatal special care units (15). The importance of bacterial and viral pathogens in causation of nosocomial infections, including surface infections, bacteremia, and pneumonia, of newborn babies has been clearly established (9, 16). Diarrhea has usually been excluded from surveillance studies of nosocomial infection, although it has been noted to be a common occurrence in newborn nurseries (16).

During 1975 the reovirus-like particle (rotavirus, duovirus, human reovirus-like agent, infantile gastroenteritis virus), now accepted as the most common cause of acute gastroenteritis in older children, was located by electron microscopy of diarrheal feces from newborn babies in the United Kingdom (7, 17) and Australia (4, 20). A pilot study undertaken in Melbourne, Australia, from December 1974 to May 1975 located rotavirus particles in diarrheal feces of babies from nurseries in five obstetric hospitals (5). The study also located a noncultivable 28-nm virus-like particle (3), which resembled both Norwalk agent (11) and the 29- to 30-nm particle that was described in feces from babies with mild diarrhea in the United Kingdom (2) and later referred to as astrovirus (18).

Local anecdotal evidence indicated that diarrhea had been a fluctuating (and perhaps seasonal) problem for many years in obstetric hospital nurseries in Melbourne. One special care nursery was selected for long-term surveillance. Feces were collected from babies with and without diarrhea and were examined by routine bacterial culture and electron microscopy. The results suggest that rotavirus infection is common during the first week of life and is an important cause of diarrhea in special care nurseries for the newborn.

PATIENTS AND METHODS

Surveillance was maintained on 913 newborn babies in the special care nursery of the Royal Women's Hospital, Melbourne, during April 1975 to June 1976. All babies studied had been admitted from the labor ward within 2 h of birth because of problems such as low birth weight, prematurity, or birth trauma, and had remained in this nursery for more than 48 h. The median duration of stay was 3 weeks.

Average daily occupancy of the nursery was 75 babies. They were housed in 11 rooms with approximately 4 m^2 of floor space per baby. Each room was separately air conditioned, there being no recirculation of air. Humidity was maintained at 55% and air temperature at 22 to 23°C. All babies were cared for by nursing staff. Mothers usually visited their babies daily and fed them. Other visitors viewed the babies through glass from outside the nursery. All babies were initially fed with cow's milk formula or with pooled expressed breast milk that was pasteurized before use.

Babies were observed daily for development of diarrhea and associated symptoms. Frequency and appearance of bowel actions were recorded, and watery stools were tested for the presence of reducing sub-

stances by the "Clinitest" method (13). This method requires fluid stools, hence control babies did not have stools tested for sugar. An infant was accepted as having diarrhea if stools became very loose and watery or were more frequent than six per day.

Feces were collected from 343 babies, most of whom were 4 to 9 days old. Two hundred thirty-eight of these babies had diarrhea of less than 48-h duration at the time of fecal collection (diarrhea group). One hundred five of the babies had no symptoms of diarrhea during their stay in the nursery (control group).

All specimens were examined by electron microscopy. After storage at +4°C for 1 to 4 weeks, 1 to 5 g of feces was homogenized with 10 ml of phosphate-buffered saline, then thoroughly mixed with 10 ml of trifluorotrichloroethane (Arklone, ICI Australia, Ltd., Melbourne). This homogenate was centrifuged at 10,000 × *g* for 20 min at 4°C. The supernatant fluid was collected and centrifuged at 100,000 × *g* for 60 min at 4°C. The deposit obtained was resuspended in 3 to 5 drops of tris(hydroxymethyl)aminomethane buffer and examined with a Hitachi electron microscope after negative staining with 1% potassium phosphotungstate (pH 7.0). All specimens were coded, and results of electron microscopy were later correlated with clinical assessment. The two noncultivable viruses frequently seen (rotaviruses and the 28-nm particle) were easily distinguished on morphological grounds (Fig. 1).

All diarrheal feces were cultured by routine methods used to detect bacterial enteric pathogens. Strains of *Escherichia coli* were subjected to slide agglutination using commercially available antisera to OB groups regarded as enteropathogenic (Baltimore Biological Laboratories, Cockeysville, Md.). Strains of *E. coli* isolated from both groups of babies during 4 months of the study were examined for ability to produce heat-labile and heat-stable enterotoxin, using Y_1 adrenal cell monolayers and intragastric inoculation of infant mice. Routine cell culture of diarrheal feces was not attempted, but fecal specimens shown to contain the 28-nm virus-like particle were inoculated into primary human kidney, primary monkey kidney, human fetal fibroblasts, and HeLa cells.

In analyses of results, statistical probabilities were calculated by the chi-square method using Yates' correction where appropriate.

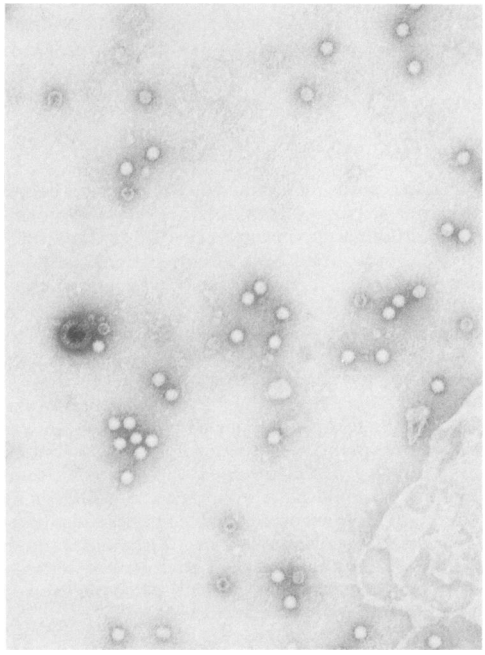

FIG. 1. *Negatively stained rotavirus and 28-nm particles in feces from a newborn baby (×58,000).*

RESULTS

Clinical observations. During the 15 months of the survey, 257 of the 913 babies developed diarrhea, an overall incidence of 28% of all babies at risk. Diarrhea occurred randomly throughout all 11 rooms of the nursery and among babies nursed in humidicribs and those in open cots.

Diarrhea was usually mild and of brief duration (1 to 2 days), and was never a direct cause of death. Vomiting, fever, and irritability were occasionally observed. In 80% of symptomatic infants, the watery green or yellow stools collected during the acute stage of the illness contained abnormal amounts of reducing sugar (>0.5%) regardless of the nature of the viral infecting agent. Treatment consisted of the substitution of normal feeds with 5% glucose in water for 12 to 24 h, followed by the gradual reintroduction of normal or lactose-free feeds. Intravenous fluid therapy for dehydration was required by less than 5% of babies. Thirty percent of babies excreting rotavirus were discharged on low-lactose feeds, compared with 20% of those not excreting rotavirus. Fewer than 20% of babies required long-term lactose-free feeds.

The incidence of diarrhea in relation to birth weight and gestational age is shown in Table 1. Gestational age of two babies was not known, and they are excluded from the table. Diarrhea

TABLE 1. *Incidence of diarrhea in babies in a special care nursery in relation to birth weight and gestational age*

Determination	No. of babies at risk	No. with diarrhea (%)
Birth weight (g)		
<2,000	230	67 (29)
2,000 to 2,999	488	139 (28)
≥3,000	195	49 (25)
Gestational age (weeks)		
<32	90	27 (30)
≥32 to <36	250	69 (28)
≥36	571	159 (28)

was as common in babies of birth weight <2,000 g (29%) and of gestational age <32 weeks (30%) as in those of higher birth weight (25 to 28%) and later gestational age (28%).

Age at onset of diarrhea varied from 28 h to 62 days (Fig. 2). Median age at onset was 4.5 days. Seventy-five percent of babies who developed diarrhea did so during the first week of life.

The monthly attack rates for diarrhea (Fig. 3) varied from a maximum 43% during winter in July 1975 to a minimum 13% during summer in December 1975 ($P < 0.005$).

Microbiological results. (i) Diarrhea group. Routine culture of diarrheal feces yielded *Salmonella havana* (1), *Salmonella bovis-morbificans* (1), *E. coli* O119:B4 (1), and *E. coli* O125:B15 (1). Enterotoxigenic strains of *E. coli* were isolated from 11 of 57 babies examined (19%).

Electron microscopy revealed rotavirus particles in feces from 127 of 238 babies with diarrhea (Table 2). Rotaviruses were detected in 50% or more of the babies with diarrhea (Fig. 3) during all but two of the months studied (April 1975, May 1976). The figures for June 1976 were excluded because the survey was discontinued midway through the month. Rotaviruses were found less often in diarrheal feces from babies of less than 32 weeks gestation (Table 3) than from more mature infants ($P < 0.005$).

Electron microscopy also revealed a 28-nm virus-like particle that failed to grow in cell cultures of primary human fetal kidney, primary monkey kidney, human fetal fibroblasts, and HeLa cells (Fig. 1). The morphology of this particle has been described elsewhere in more detail (6). It is morphologically similar to Norwalk agent. The 28-nm particle was detected in feces of 64 of 238 babies with diarrhea, 36 of whom were simultaneously excreting rotavirus (Table 2). There was a marked variation in monthly detection of this particle (Fig. 4), ranging from none detected during October and November 1975, to a maximum 50% detected in May 1976.

(ii) Control group. No enteric bacterial pathogens were isolated from this group of babies. Enterotoxigenic strains of *E. coli* were isolated from 4 of 22 babies examined (17%).

Rotavirus particles were seen in feces from 37 of 105 control babies (Table 2), including 11 of 24 babies examined during August 1975, 9 of 27 during September 1975, 8 of 13 during October 1975, and 8 of 23 babies during June 1976. The 28-nm particle was seen in 16 control babies, 6 of whom were simultaneously excreting rotaviruses.

(iii) Viruses in diarrheal feces in relation to diet: Of 210 babies with diarrhea, 188 had received artificial feeds (mainly cow's milk), and

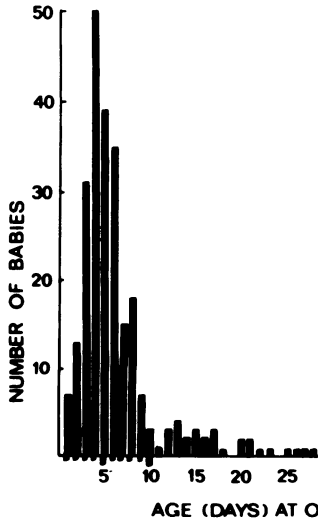

FIG. 2. *Age of 257 newborn babies at onset of diarrhea.*

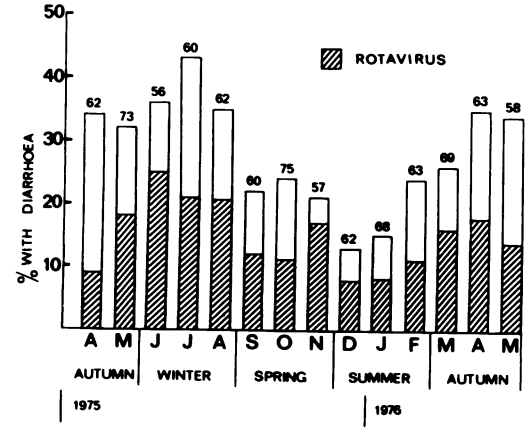

FIG. 3. *Monthly incidence of diarrhea and detection of rotavirus in diarrheal feces from newborn babies in a special care nursery. Figures above the columns are the number of babies at risk during each month.*

TABLE 2. *Electron microscopic detection of two viruses in fecal extracts from babies admitted to a special care nursery (April 1975 to June 1976)*

Determination	No. of babies examined	Rotavirus (%)	28-nm virus (%)	Rotavirus and 28-nm virus[a]
Diarrhea	238	127 (53)	64 (27)	36
No diarrhea	105	37 (35)	16 (15)	6

[a] These figures are included in the total for each virus separately.

TABLE 3. *Electron microscopic detection of two viruses in neonates with diarrhea related to birth weight and gestational age*

Determination	No. of babies examined	Rotavirus (%)	28-nm virus (%)	Rotavirus and 28-nm virus[a]
Birth weight (g)				
<2,000	59	24 (41)	7 (12)	3
2,000 to 2,999	130	77 (59)	42 (32)	24
≥3,000	49	26 (53)	15 (31)	9
Gestational age (weeks)				
<32	22	6 (27)	4 (18)	1
≥32 to <36	68	30 (44)	10 (15)	6
≥36	148	91 (61)	51 (34)	29

[a] These figures are included in the total for each virus separately.

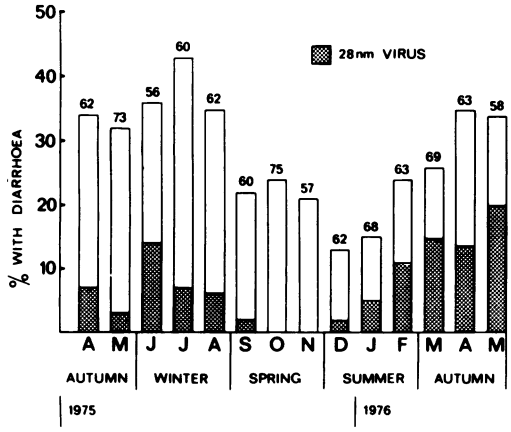

FIG. 4. *Monthly incidence of diarrhea and detection of a 28-nm virus-like particle in diarrheal feces from newborn babies in a special care nursery.*

22 had been given pooled and pasteurized expressed breast milk. Fifty-four percent of babies fed artificial formulas (102/188) excreted rotavirus, compared with 41% (9/22) fed breast milk. Twenty-eight percent of babies fed artificial formulas (53/188) excreted the 28-nm particle, compared with 9% who were fed breast milk.

DISCUSSION

Diarrhea was an endemic problem among newborn babies admitted to one special care nursery in Melbourne, Australia, during 15 months of surveillance. Symptoms developed in 28% of all babies at risk, with a peak incidence of 43% during the winter month of July 1975. Most babies developing diarrhea did so during the first 10 days of life, the youngest being only 28 h old.

Many babies in special care nurseries are physiologically and immunologically immature, as well as critically ill. Despite this, diarrhea was not more frequent nor more severe in infants of very low birth weight or very early gestational age than in more mature babies. Early diagnosis and treatment with appropriate oral fluids may have contributed to the mildness and brevity of symptoms in most babies. However, diarrhea was severe in some babies and was a potential threat to life in a small proportion of babies (<5%), who required intravenous therapy to replace fluid losses. There was no apparent relationship between severity of diarrhea and the infectious agent present in feces. Clinical differences, if any, between infections due to rotavirus and those due to toxigenic *E. coli* require more detailed study.

Rotavirus infection was present in 53% of all babies with diarrhea. The monthly rate of detection remained almost constant throughout the study. A more sensitive technique than electron microscopy might have shown the true incidence to be even higher. The lower rate of rotavirus detection in babies of less than 32-weeks gestation suggests that physiological immaturity of the gut, especially the relatively late development of lactase (10), may contribute directly to development of diarrhea in this group.

Previous studies have queried the role of rotaviruses as a cause of diarrhea in the human newborn (19, 22), while accepting that these viruses cause serious gastroenteritis in older children (1) and in the newborn of other animal species (23). This study showed an increase in rotavirus infection in babies with diarrhea (53%), compared with a control group (35%), and supports our belief that rotavirus infection was the main cause of endemic diarrhea in this nursery. Other recognized enteric pathogens, including enterotoxigenic *E. coli,* were isolated from only a minority of the babies. The 28-nm particle could not be conclusively shown to be an enteric pathogen. There was no statistical difference between incidence of infection in patients and controls, and the excretion pattern did not usually coincide with symptoms of diarrhea (6). The increased incidence of infection during winter months might have potentiated disease caused by rotavirus, as has been observed in mixed infections with rotavirus and a 28-nm particle in gnotobiotic piglets (23).

We found a much lower incidence of asymptomatic rotavirus infection than has previously been reported in similar studies. Of babies infected with rotavirus in this special care nursery, 77% (127/164) had diarrhea, compared with 28% (19) and 8% (22) of full-term babies in two other studies. This is partly explained by differences between these studies in definition of diarrhea and in clinical assessment of patients. Totterdell

et al. (22) defined diarrhea rigidly as "the passage of a dozen or more loose and often offensive stools in 24 hours." Murphy et al. (19) did not define diarrhea, and classified symptoms as diarrhea, "loose" or "mucousy" stools, or "minor intestinal symptoms," Careful clinical assessment is crucial, since asymptomatic excretion of rotavirus precedes onset of symptoms in the neonate (6), and symptoms may be very brief. Differences in incidence of asymptomatic infection could also be explained by variations in virulence of rotavirus strains and by differences in host resistance in healthy full-term babies as compared with babies requiring special care.

The seasonal pattern of diarrheal infection in newborns has been previously documented (14, 21), but cannot be explained by variations in temperature and humidity in this climate-controlled nursery. Monthly incidence of diarrhea shows a close correlation with monthly hospital admissions of older children with rotavirus gastroenteritis during a previous year in the same community (Fig. 5). This implies that rotavirus infection is repeatedly introduced into newborn nuseries during winter when the community incidence is high. If this hypothesis is correct, then adults must have been the source of infection, since children were not permitted as visitors to the nursery. Adult contacts of children with symptomatic rotavirus infection often excrete rotavirus asymptomatically (11). Thus parents and nursery attendants could have been a source of infection. Infection from asymptomatic mothers during labor might account for the very early onset of symptoms in some babies.

The results of this survey demonstrates the frequency of rotavirus infection in newborn nurseries and the importance of this enteric pathogen as a cause of endemic diarrhea in neonates. It is now necessary to delineate clinical symptoms other than diarrhea, to identify the factors modifying the clinical effects of rotavirus infection in neonates, and to follow the development of immunity after natural infection in the newborn child.

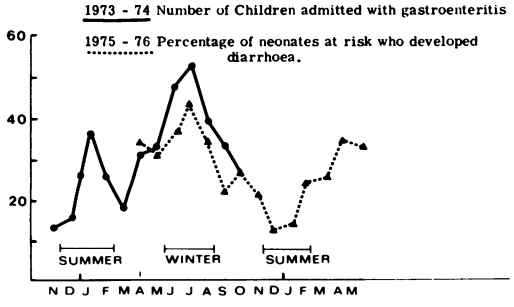

FIG. 5. *Seasonal comparison of the percentage of neonates at risk who developed diarrhea with the number of admissions of older children with gastroenteritis.*

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